THE ACTIVITY PATTERN OF TWO PEROXISOMAL OXIDASES IN THE RAT REPHRON

Michel LE HIR and Ulrich C. DUBACH

Medizinische Universitäts-Poliklinik and Department Forschung, Kantonsspital, 4031 Basel, Switzerland

Received 19 March 1981

1. Introduction

The enzyme common to all peroxisomes, catalase, is associated in these organelles with H_2O_2-producing oxidases [1]. The production of H_2O_2 is partly coupled in vivo to the peroxidatic activity of catalase [2–4]. The elucidation of the specific role of the peroxisomes has been undertaken mainly with the liver where they are densely distributed. Studies of other organs might help to find out new aspects of the function of the peroxisomes. Thus it may be useful to see if other tissues are suitable for the type of investigations carried out on the liver. For instance the kidney, particularly in the proximal tubule, contains numerous peroxisomes [5,6], suggesting that this organ might be an interesting object for study. Because of the histological complexity of the kidney the assay of enzymes in defined segments of the nephron is an important, although often preliminary, step for the comprehension of their function.

Using a microassay for H_2O_2 [7], we measured the activity of D-amino acid oxidase (DAAO) and L-α-hydroxyacid oxidase (LHAO) in microdissected segments of the nephron of the rat. The results show that the role of the peroxisomes in the cells of the proximal tubule might be as important as in the hepatocytes.

2. Materials and methods

D-Amino acid oxidase, crystallized catalase and FAD were obtained from Boehringer (Mannheim); D-proline, L-α-hydroxyisocaproate, 3-amino-1,2,4-triazole and BSA from Sigma. Male Wistar rats weighing 200 g, fed a standard laboratory diet up to killing, were killed by decapitation. The kidneys were frozen in liquid nitrogen. 14-μm thick sections were cut in a cryostat. They were freeze-dried and stored at −40°C. Segments of the nephron were dissected out of these sections and weighed on a quartz fiber balance.

Single pieces of proximal tubules (5–30 ng) were analyzed separately. For glomeruli, distal and collecting tubules, 2–4 pieces were pooled (30–80 ng). The microassays for the oxidases are based on the inhibition of exogenous catalase by aminotriazole in the presence of a source of H_2O_2. Under appropriate conditions the inhibition of catalase is proportional to the activity of the H_2O_2-producing oxidase [7]. Catalase was assayed spectrophotometrically and its activity expressed as the first-order rate constant k [8]. The incubation media for the microassays are:

DAAO: 35 mM D-proline, 125 mM aminotriazole, 5 μM FAD, 1 mM EDTA, 0.05% BSA, 0.1% Triton X-100 and catalase (k = 1.6) in 0.1 M pyrophosphate buffer pH 8.3.

LHAO: 4 mM α-hydroxyisocaproic acid, 100 mM aminotriazole, 1 mM EDTA, 0.05% BSA, 0.1% Triton X-100 and catalase (k = 1.6) in 0.1 M pyrophosphate buffer pH 8.3.

In order to convert the inhibition of catalase into units of activity of the oxidases standards were run parallel to the samples in the microassay. They consisted of a known activity of DAAO or LHAO contained in the same substrate medium as for the samples. A commercial enzyme was used in the standard for DAAO and a freeze-dried mitochondrial fraction of a homogenate of kidney cortex for LHAO. The activities of the oxidases used in the standards were measured by a microassay shortly before dilution in the incubation medium for the microassay. They are expressed as units (μmol . min^{-1}). Details of the procedure for the microassay were as in [9].

3. Results

The activities of DAAO and LHAO in dissected segments of the nephron are shown in fig.1. Since the S.D. of the residual activity of catalase in the blanks (4 tubes without oxidase) was in each experiment less than 5%, an inhibition of catalase exceeding 10% in a sample was considered as a safe indication for activity of the oxidase. Single proximal segments weighing as little as 5 ng gave more than 20% inhibition of catalase in the medium for DAAO. In the case of LHAO large pieces of proximal tubule (more than 15 ng) gave more than 10% inhibition of catalase. With glomeruli and distal straight parts the activities of catalase in the samples were never significantly different from the activities in the blanks. With distal convolutions the inhibition of catalase with some samples exceeded 10% in the medium for DAAO but not in the medium for LHAO. Thus, according to our criterion of 10% inhibition, we cannot prove the presence of HAOX in the distal convolution. With cortical collecting tubules 10% inhibition of catalase was obtained in nearly all samples in the medium for DAAO, and in some samples in the medium for LHAO.

4. Discussion

The principle of our assay for H_2O_2-producing oxidases is that catalase is inhibited by aminotriazole

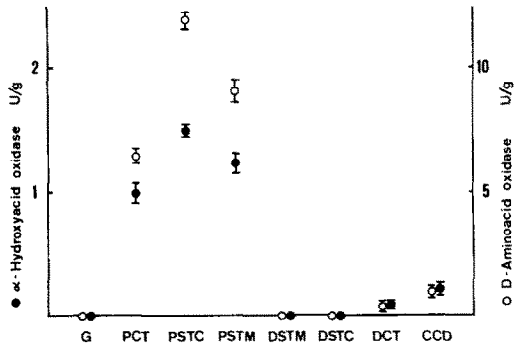

Fig.1. Activities of DAAO and LHAO in microdissected segments of the nephron. Each point represents the mean of six rats ± S.E. G, glomerulus; PCT, proximal convoluted tubule; PSTC, proximal straight tubule, cortical part; PSTM, proximal straight tubule, medullary part; DSTM, distal straight tubule, medullary part; DSTC, distal straight tubule, cortical part; DCT, distal convoluted tubule; CCD, cortical collecting duct.

in the presence of a source of H_2O_2 [7]. Production of H_2O_2 through microsomal or mitochondrial systems should be very low without addition of pyridine nucleotides or succinate [10]. Accordingly, inhibition of catalase in our assay was obtained only in the presence of a substrate for a peroxisomal oxidase. To our knowledge the present micromethod is the only one to be sensitive enough for the assay of the production of H_2O_2 with single dissected segments of the nephron. DAAO and LHAO have a similar distribution along the nephron (fig.1). The maxima are found in the cortical straight part of the proximal tubule, where the density of peroxisomes is highest [5]. Failure to detect activity in the straight part of the distal tubule does not conflict with the description of peroxisomes in this segment. Peroxisomes have been identified in the distal tubule only by the staining of catalase. In the straight part they were not detected [5] or appeared very scarcely [6,11,12]. Accumulation of catalase-positive organelles occur in a population of cells in the distal convolution [6,11, 12]. The figures published in the 3 papers cited suggest to us that those cells are not typical distal tubular cells, but rather represent principal cells, the main type of cells in the collecting duct occurring also in the distal convolution of some species [13]. Our interpretation is supported by the demonstration of DAAO and LHAO in the collecting tubule. Our results diverge in part from a previous report on the distribution of DAAO in the rat nephron [14]. In the latter study the production of pyruvate from D-alanine was measured. The difference of activity between the various parts of the proximal tubule was less than what we observed. The major discrepancy, however, is found in the distal convolution. In the study cited the activity measured in that segment was 30% of that measured in the proximal straight tubule. In view of the scarcity of peroxisomes in that segment, and because the assay was not founded upon the production of H_2O_2, it may be questionable if the activity measured was due to a peroxisomal oxidase. However, DAAO is an induced enzyme [15], and therefore it cannot be excluded that under certain conditions the enzyme may reach high activity in the distal tubule.

The activity of DAAO in the proximal tubule is several fold that measured with the same method in the liver [9]. The activity measured for LHAO is also higher in the proximal tubule than in the liver. However, the validity of the comparison in the case

of LHAO is limited by the fact that the kidney enzyme shows a substrate specificity differing from that of the liver enzyme [16,17]. We used glycolate as substrate for the liver [11] and α-hydroxyisocaproate for the kidney. The kidney is in second position of 7 organs from the guinea pig in regard to the content of the peroxisomal (H_2O_2-producing) palmitoyl-CoA oxidase [18]: the activity in the kidney is more than half of that in the liver. In preliminary experiments we found a similar ratio in the rat. If the palmitoyl-CoA oxidase is essentially concentrated in the proximal tubule like DAAO and LHAO, then the part played by the peroxisomes in the oxidative metabolism is expected to be of similar importance in the proximal tubular cells as in the hepatocytes. Thus, the spectrophotometric method used with the liver for the analysis of the metabolism of H_2O_2 [2–4] should be applicable to suspensions of proximal tubules [19].

Acknowledgement

Financial support by the Swiss National Foundation (Grant 3.900–0.79) is gratefully acknowledged.

References

[1] De Duve, C. and Baudhuin, P. (1966) Physiol. Rev. 46, 323–357.
[2] Sies, H. and Chance, B. (1970) FEBS Lett. 11, 172–176.
[3] Oshino, N., Oshino, R. and Chance, B. (1973) Biochem. J. 131, 555–567.
[4] Oshino, N., Jamieson, D., Sugano, T. and Chance, B. (1975) Biochem. J. 146, 67–77.
[5] Beard, M. E. and Novikoff, A. B. (1969) J. Cell. Biol. 42, 501–508.
[6] Roels, F. and Goldfischer, S. (1979) J. Histochem. Cytochem. 27, 1471–1477.
[7] Le Hir, M. (1980) Anal. Biochem. 102, 233–236.
[8] Aebi, H. (1974) in: Methoden der Enzymatischen Analyse (Bergmeyer, H. U. ed) pp. 713–724, Verlag Chemie Weinheim.
[9] Le Hir, M. and Dubach, U. C. (1980) Histochemistry 69, 95–99.
[10] Chance, B., Sies, H. and Boveris, A. (1979) Physiol. Rev. 59, 527–605.
[11] Chang, C. H., Schiller, B. and Goldfischer, S. (1971) J. Histochem. Cytochem. 19, 56–62.
[12] Novikoff, A. B., Novikoff, P. M., Davis, C. and Quintana, N. (1972) J. Histochem. Cytochem. 20, 1006–1023.
[13] Kaissling, B. (1980) Cell Tiss. Res. 212, 475–495.
[14] Chan, A. W. K., Perry, S. G., Burch, H. B., Fagioli, S., Alvey, R. T. and Lowry, O. H. (1979) J. Histochem. Cytochem. 27, 751–755.
[15] Lyle, C. R. and Jutila, J. W. (1968) J. Bacteriol. 96, 606–608.
[16] Nakano, M. and Danowski, T. S. (1966) J. Biol. Chem. 241, 2075–2083.
[17] Ushijima, Y. and Nakano, M. (1969) Biochim. Biophys. Acta 178, 429–433.
[18] Small, G. M., Brolly, D. and Connock, M. J. (1980) Life Sci. 27, 1743–1751.
[19] Balaban, R. S., Soltoff, S. P., Storey, J. M. and Mandel, L. J. (1980) Am. J. Physiol. 238, F50–F59.